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A CONTRIBUTION TO THE PARASITOLOGY OF TRYPANOSOMIASIS

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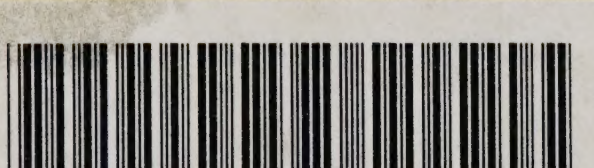
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A CONTRIBUTION TO THE PARASITOLOGY OF TRYPANOSOMIASIS.*

S. B. WOLBACH AND C. A. L. BINGER.

(*From the Department of Bacteriology, Harvard University Medical School.*)

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INTRODUCTION. — The pathology of natural and experimental trypanosomiasis has been studied by Mott,[1] Thomas and Breinl,[2] Pettit,[3] Sauerbeck,[4] Mole,[5] and others. Mott especially has done classical work on the microscopic changes found in sleeping sickness and dourine. More recently the work of Warrington Yorke[6] has cast some light on the reaction of cornea and skin to the presence of trypanosomes. A review of the more important literature is given by Laveran and Mesnil.[7]

Lesions of the central nervous system have been investigated perhaps more carefully than any others. Injection and infiltration of the méninges are always present, even when not macroscopically demonstrable. This condition is accompanied in chronic cases by myelitis and encephalitis, characterized by proliferation and overgrowth of neuroglia cells, by perivascular infiltration of lymphoid and plasma cells, and by proliferation of endothelial cells. The changes found in the nervous system in sleeping sickness, Mott thinks, very closely resemble those found in syphilis except that proliferative endarteritis, common in syphilis, is absent in sleeping sickness.

In addition to cerebro-spinal lesions, enlargement of lymphatic glands, spleen, and liver is frequently seen in patients

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who have died of sleeping sickness and in experimentally infected animals. Microscopic examination of these organs and of lungs, kidneys, heart, pericardium, and alimentary canal reveals lesions characterized in general by lymphoid, plasma, and endothelial cell proliferation.

From the pathological findings some discussion has arisen as to the action of the organism in the body of the host. The chronic inflammatory changes have been attributed to the presence of trypanosomes as well as to absorption of toxins. According to Laveran and Mesnil[7] (p. 397): "Trypanosomes even when numerous in the blood or in smears of tissues, such as the lymphatic glands during life, are nearly always very scanty in sections of organs and tissues." And Mott[1] (Reports of Sleeping Sickness Commission, No. VII., p. 12) states that "the microscopic evidence of the existence of trypanosomes in the sections of glands is not more satisfactory than the evidence of their existence in perivascular and meningeal infiltration of the nervous tissue." Not until the work of Yorke[6] had flagellate forms in any considerable numbers been demonstrated in tissues. Yorke found large numbers of trypanosomes in the edematous interstitial spaces of the skin of rabbits and corneæ of goats experimentally infected with T. rhodesiense.

The purpose of the work upon which this paper is based was to study the distribution of trypanosomes in tissues and to ascertain the factors concerned in the production of the lesions of trypanosomiasis. It was stimulated by the acquisition of a reliable technic for the staining of trypanosomes in tissues.

The generally negative results of all work directed towards the production of immune phenomena led us to believe that the connection between the lesions and the parasite must be a very direct one in trypanosomiasis. Incidentally we hoped to acquire some new facts regarding the biology of trypanosomes in the vertebrate host.

TECHNIC. — The attempt to apply Romanowsky staining

to tissues was begun by one of us in 1910. The method used by Wright[8] for staining blood platelets in bone marrow gave indifferent results with trypanosomes in tissues. Attempts to apply Giemsa's stain gave indications of success when the publication by Giemsa appeared in 1910.[9] His method has given very good results, and, subject to a few minor modifications and strict attention to proper fixation and thinness of sections, has enabled us to stain trypanosomes in tissue with the same certainty that the tissue elements are stained. The modifications in treatment of the sections are the removal of the corrosive crystals with very weak alcoholic solutions of iodine, and the differentiation of the stained sections with a strong solution of colophonium in acetone[10] instead of the xylol-acetone mixtures recommended by Giemsa. The steps are as follows:

1. Fix tissues immediately after death of the animal in saturated solution of pure corrosive sublimate in distilled water, two parts; absolute alcohol, one part. The slices of tissue should not be over two millimeters thick. Forty-eight hours are required for good fixation, but the tissues may remain in the fixative for several weeks without harm.

2. Dehydrate in alcohol, clear in oil of cedar wood, and embed in paraffin. The tissues must not be handled with metal instruments until they have been cleared in oil of cedar wood. If trimming is necessary it should be done just before the tissues are placed in the paraffin bath. The sections should not be over four microns thick; two microns give better results, as the staining solution penetrates poorly. We have used two micron sections for the greater part of our work.

3. Remove paraffin with xylol, and follow by graded alcohols and water.

4. Treat with weak alcoholic iodine solution (saturated solution of iodine in ninety-five per cent alcohol, three to four cubic centimeters; seventy per cent alcohol, one hundred cubic centimeters) for fifteen to twenty minutes.

5. Treat with eighty per cent alcohol until the yellow color is removed, wash in distilled water, and then place into

a .5 per cent aqueous solution of hyposulphite of sodium for ten minutes, wash in running water at least five minutes, and rinse in distilled water.

6. Stain in freshly diluted Giemsa solution four to twelve hours. The dilution should be sixty to eighty drops of the stain to one hundred cubic centimeters of water. The stain is replaced twice by fresh mixtures during the first hour.

The water used for diluting the stain must be absolutely free from acids. A trace of carbonic acid gas or of volatile organic acids spoils the staining (Giemsa). The distilled water should be tested and corrected in the following manner: In each of four flasks place three hundred cubic centimeters of the water and add a one per cent solution of sodium carbonate, one drop to the first flask, two drops to the second, and so on. Then take ten cubic centimeters from each flask in clean test-tubes and add to each two or three drops of a freshly prepared solution of hematoxylin in absolute alcohol. This solution should be pale yellow to almost colorless.

The water with the right reaction will take on a violet tinge in four to five minutes. The test-tubes should be placed against a white background in order to read the reaction easily.

7. Transfer the sections for differentiation directly from the staining mixture, or after a rapid passage through distilled water, into two changes of colophonium fifteen grams, acetone one hundred cubic centimeters. The slides should be treated individually, and the differentiating fluid renewed as soon as the colophonium precipitated by the water fails to dissolve rapidly. Differentiation takes place rapidly; fifteen to thirty seconds is usually sufficient. Should a deeper staining with blue be desired, the amount of colophonium should be reduced.

8. Pass the sections rapidly through acetone seventy cubic centimeters, xylol thirty cubic centimeters; pure xylol, and oil of cedar wood. Mount in oil of cedar wood.

This method gives the same staining of trypanosomes that

is obtained in film preparations. If fixation has been immediate and the sections cut not thicker than two microns, the flagellum, undulating membrane, cytoplasm, nucleus, and blepharoplast are all clearly defined. In thicker sections the flagellum is not always stained. It is easy to recognize divisional forms by the presence of more than one nucleus and blepharoplast. Where we note the identification of degenerated forms, "rounded forms," and forms within phagocytes, the demonstration of nucleus and blepharoplast at least has always been made. This rule is necessary because remains of tissue nuclei within phagocytes may otherwise be mistaken for trypanosome remains. Blood platelets are well stained and likewise may be mistaken for trypanosome nuclei, especially as they are taken up by endothelial cells in lymph nodes and spleen.

For the study of the lesions and recognition of cells, parallel sets of tissues were fixed in Zenker's fixative, embedded in paraffin, and stained with eosin-methylene-blue, Mallory's connective tissue stain, and Mallory's phosphotungstic acid hematein. The last stain was used chiefly for the recognition of fibroblasts, which are identified by the delicate fibroglia fibrils. The phosphotungstic acid hematein stain usually stains the nucleus and sometimes the blepharoplast and the flagellum of trypanosomes in sections, though not strongly enough to make the method reliable (Fig. 14). The eosin-methylene-blue method now and then stains trypanosomes in sections very well (Fig. 34), but constant results cannot be obtained even with the same tissue. In thin sections of well-fixed tissue the nuclei are usually stained, though faintly.

MATERIAL. — The amount of material used is not large, but the constancy of the findings contraindicated what seemed to us to be a needless repetition. The two strains of trypanosomes were brought from the Runcorn laboratory of the Liverpool School of Tropical Medicine; a strain of Trypanosoma brucei, originally from Uganda, and a strain of Trypanosoma gambiense from the French Congo.

White rats, guinea-pigs, and monkeys were used. With Trypanosoma gambiense, sets of tissues from four white rats, six guinea-pigs, and three monkeys (Macacus rhesus) were studied. With Trypanosoma brucei there were four white rats and three guinea-pigs. The animals utilized, with the exception of the monkeys, were selected from larger series inoculated for other purposes. The duration of the disease varied among the individuals of each series; but it is our intention to describe only those findings which seem to be constantly associated with trypanosome infection.

Besides the laboratory animals a few naturally infected animals were studied from tissues preserved in the Gambia. These include: three birds, a horn-hill (Lophoceros nasatus) and two doves (Turtus senegalensis); two lizards (Agama colonorum); three rats (Epymis); and an Ayrshire bull, imported into the Gambia. The descriptions of the trypanosomes and the identification of the infected animals are published in another paper.[11]

Of the naturally infected animals, all of which showed no evidence of disease, only one native to the Gambia — an immature rat with T. lewisi — shows lesions. The imported bull, which was almost moribund when killed, but which had no subcutaneous edema nor petechiæ in the serous membranes, shows histologically the same lesions and distribution of trypanosomes that were found in the experimental animals. The type of trypanosome [11] was T. dimorphon (Dutton and Todd). The gross anatomical changes were enlarged soft spleen, pale fat of loose texture but not edematous, a few small hemorrhages at the attachment of the meso-colon, and enlarged glands. Some small hemorrhagic foci in the lungs were possibly due to an intravenous injection of one gram of salvarsan given twenty-two hours before the animal was shot.

PATHOLOGY AND DISTRIBUTION OF TRYPANOSOMES IN TISSUES. — No external lesions, other than emaciation, were seen in any of the experimental animals except in one monkey and six guinea-pigs infected with T. gambiense

Three only of these six guinea-pigs were made use of for the purposes of this report. The monkey which was inoculated on April first developed edema of the eyelids on May second, and rapidly became moribund. When chloroformed on May fourth both eyes were closed by the marked edema of the lids, and there was a slight excoriation of the right eyelid. There was a dry, scaly lesion on the bridge of the nose. The six guinea-pigs with external lesions were inoculated, four on November first, and two on December sixteenth. In none of these guinea-pigs were trypanosomes found in the blood. The first four were examined at intervals after the first week, as they were intended for class purposes. The second lot of two were examined occasionally during the last month.

The strain of T. gambiense, since its removal from Runcorn in July, 1911, had, up to this time, been carried on in white rats. The lesions began about three months after inoculation as edema of the eyelids, conjunctivitis, edema about the anus and genitalia, and induration of the skin of the back in the median line. Loss of hair occurred during the fourth week from the eyelids and surrounding skin, about the genitalia, and from the back. In several guinea-pigs there was marked brawny edema of the inguinal folds. Many trypanosomes were found in juice obtained by puncture of the enlarged inguinal lymph nodes and in the lymph obtained by excoriating the surface of the edematous skin during the third month. Toward the end of the fourth month marked edema of the ears, complete closure of the eyelids, and in three instances ulceration of the back and skin of the anal region appeared (Figs. 1, 2, and 3). The three guinea-pigs examined were killed when their condition made it evident that they could not survive the infection for a much longer period. The other three died during the first half of the fifth month of the infection.

Lesions of this sort have been produced in guinea-pigs with other trypanosomes: T. brucei [7] and T. hippicum,[12] and in other animals with T. gambiense and other trypanosomes.[6] This we believe to be the first record of extensive gross

external lesions in guinea-pigs infected with T. gambiense. The long continued passage in rats was probably responsible for the change in virulence of the strain of trypanosome which made the type of infection possible.

PATHOLOGY AND DISTRIBUTION OF TRYPANOSOMES IN TISSUES.

Heart: No gross changes were seen. The heart was preserved in a few of our animals only. Microscopic lesions were found in the hearts of two guinea-pigs inoculated with T. gambiense on November first, 1911, and killed on March twenty-fifth and March twenty-ninth, 1912. These lesions are very minute infiltrations of the endocardium and myocardium beneath with endothelial, lymphoid, and plasma cells; these were seen in the Zenker fixed tissues of both cases, and in the Giemsa stained tissues of but one. No trypanosomes were found in this case.

Extensive infiltration of the endocardium and of the intermuscular septa of the myocardium is present in the sections of the heart of the Ayrshire bull, which was infected with T. dimorphon. The endocardial lesions are focal and consist of collections of endothelial cells, lymphoid, and plasma cells. The lesions of the myocardium consist of diffuse edema of the intermuscular connective tissue septa, and infiltration similar to that in the endocardium. The infiltration extends irregularly between the muscle fibers. There are also numerous fibroblasts. The lymphatics are injected and many are filled with large endothelial cells.

Trypanosomes and trypanosome nuclei are very numerous in the lesions from both locations. These are found in the connective tissue of the intermuscular septa, between the muscle fibers, in the lymphatics, and within phagocytic endothelial cells (Figs. 27 and 28).

Lung: No gross changes were noted. It is probable that some were overlooked. The lesions which are constant are infiltration of the connective tissue about vessels and bronchi, and proliferation and accumulation of endothelial

cells in the capillaries of the alveolar walls. Hyperplasia of the lymphoid tissue present near bronchi is almost constant.

The infiltrative cells in the connective tissue consist of endothelial, lymphoid, and plasma cells. The fat tissue, found in one instance about the great vessels and primary bronchi, is involved with the connective tissue.

The accumulations of endothelial cells in the capillaries of the alveolar walls may be so marked in foci as to cause complete obliteration of the lumina and very marked thickening of the walls. Giant cells are found within the capillaries and in the alveolar spaces. In two instances in a T. gambiense guinea-pig of seven weeks' duration and a T. brucei white rat, also of seven weeks' duration, there are some foci of solidification due to packing of the capillaries and alveoli with endothelial cells and giant cells (Figs. 9 and 10). The lungs of the naturally infected bull and immature rat also show intracapillary proliferation of endothelial cells. Mitotic endothelial cells are common in the capillaries in the lungs of all animals examined (monkeys, guinea-pigs, and rats, Fig. 22). The endothelial cells are often finely vacuolated, even when there are no inclusions. Similarly vacuolated endothelial cells are found in the sinuses of spleen and lymph nodes.

Trypanosomes are found in the capillaries, in lymphatics, in the lesions of the peri-vascular and peri-bronchial connective tissue and fat, and in the connective tissue of the pleura (Figs. 16, 18, 19, and 30). Remains of trypanosomes, which usually consist of a nucleus and blepharoplast lying within a vacuole, are common in endothelial and giant cells. An occasional round or oval form with cytoplasm (stained a deep blue), nucleus, and blepharoplast is found in the endothelial cells (Fig. 19).

Spleen: The gross changes noted are enlargement, dark red color, and occasional softening. The constant microscopic findings are dilatation of the sinuses with blood and endothelial cells, increase in size of the lymphoid follicles, and increase of endothelial cells in the reticular tissue.

Giant cells occur in the sinuses of the spleens of guinea-pigs. The endothelial cells are often finely vacuolated. Mitotic endothelial cells are common. Lesions of the capsule are common and consist of foci infiltrated with endothelial cells, and lymphoid, and plasma cells.

Trypanosomes are found in the lesions of the capsule, in the sinuses and meshes of the reticular tissue. Trypanosome nuclei and blepharoplasts are common in endothelial cells and in giant cells (Fig. 7). The latter, when present, usually contain large numbers of trypanosome nuclei. Rounded and oval forms consisting of deep blue stained cytoplasm, a nucleus and blepharoplast, have also been found in giant cells in the spleen. In one instance (T. gambiense monkey — December 16th, 1911, to February 9th, 1912), trypanosomes were found in large numbers in the edematous and infiltrated connective tissue at the hilum of the spleen (Fig. 26).

Liver: No gross changes, other than variation in blood content, were noted. Proliferation of the endothelial cells lining the sinusoids is present in the livers of all the experimental animals and in the naturally infected rat and bull. In all cases greatly enlarged endothelial cells are found attached to the sinusoid walls and in many instances free in the lumena (Figs. 20 and 29). These cells are phagocytic and contain trypanosome remains, red blood corpuscles and polymorphonuclear leucocytes. There are occasional endothelial cells, containing trypanosome nuclei, which are packed with polymorphonuclear leucocytes. In one instance (a monkey inoculated on December 16th, 1911, with T. gambiense, and killed February 9th, 1912), there are small focal necroses evidently caused by packing of the sinusoids with endothelial cells so laden. These necroses are very sharply defined. The liver cells immediately adjacent contain hyaline droplets and are invaded by polymorphonuclear leucocytes, but the cells and the staining reaction of the surrounding liver tissue are not abnormal.

A lesion which is almost constant is infiltration of the

connective tissue of portal canals with endothelial, lymphoid and plasma cells; and in numerous instances the infiltration extends into the liver tissue. A similar infiltration is often found around the central veins of the lobules.

Trypanosomes are found in capillaries, lymphatics, in the lesions of the portal spaces and around central veins, and free and attached to endothelial cells in the sinusoids. Trypanosome nuclei and blepharoplasts are found in phagocytic endothelial cells, in the lesions, and in the sinusoids (Figs. 11, 15, and 29).

Gastro-intestinal tract: No gross changes were observed. Tissue from the gastro-intestinal tract was saved from a few cases only. Extensive lesions consisting of edema and infiltration of the submucosa were found in the stomach, duodenum, and colon of a rat inoculated with T. gambiense on December thirtieth, 1911, and killed January seventeenth, 1912. Slight lesions in the subperitoneal connective tissue of the colon were found in a T. gambiense guinea-pig infected from November first, 1911, to March twenty-ninth, 1912, and in the submucosa of the stomach of a T. gambiense monkey infected from December sixteenth, 1911, to January eleventh, 1912.

Trypanosomes were found in the lesions from these three cases. They were very abundant in the rat (Fig. 27).

Kidney: No gross changes were observed. The constant microscopic lesion is the proliferation and accumulation of endothelial cells in the capillaries of the glomerular tufts. In marked cases the tufts are almost bloodless and mitotic figures are numerous (Fig. 21). In one case of long infection (November 1, 1911, to March 29, 1912), in a T. gambiense guinea-pig, there is slight fibrosis of the glomerular tufts, most marked at the base and extending outwards into the capillaries, just as in the late lesions of intracapillary glomerulo-nephritis of man. Swelling and proliferation of the capsular epithelium is present in a few cases of T. gambiense and T. brucei infection.

A common, though not constant, form of lesion is small areas of infiltration in the interstitial connective tissue in the neighborhood of glomeruli and occasionally in regions remote from glomeruli. The invading cells are, as elsewhere, endothelial cells, lymphoid cells, and plasma cells. In large foci the tubules are compressed, their cells show hyaline degeneration and are invaded by polymorphonuclear leucocytes. Similar lesions are found in the connective tissue at the pelvis of the kidney.

Trypanosomes are found in the lesions (Fig. 17) and in blood vessels, and most abundantly in the capillaries of glomeruli. Trypanosome remains, nuclei, and blepharoplasts are found in phagocytic endothelial cells.

Adrenal: No gross changes or microscopic lesions were found in any of the experimental and naturally infected animals. Infiltration of the capsule of the adrenal was seen twice, and there are many trypanosomes in these lesions.

Lymph nodes: Enlargement of the superficial and deep lymph nodes was constant in our experimental animals and in the naturally infected bull. In rapidly progressing T. brucei infections in rats and guinea-pigs the superficial lymph nodes, axillary and inguinal, were occasionally pink or red.

The constant microscopic changes are distension of the sinuses with endothelial cells, increase in size of the lymphoid follicles, and an endothelial cell infiltration of the reticular tissue.

The sinuses are often tightly packed with large endothelial cells many of which contain trypanosome remains and red blood corpuscles; rarely, an endothelial cell with several nuclei is found in a peripheral sinus. The endothelial cells are often finely vacuolated (Fig. 12).

The capsules and adjacent areolar and fat tissue are often infiltrated with endothelial, lymphoid and plasma cells, and these lesions always contain great numbers of trypanosomes.

Trypanosomes are found in the sinuses and in the reticular

tissue. Trypanosome remains occur in large numbers in phagocytic endothelial cells. Round or oval forms with dense blue-stained cytoplasm, nucleus, and blepharoplast are occasionally found (Fig. 12).

Bone marrow: The constant gross change seen in the marrow of the femur was change of color to dark red and loss of fatty consistency.

Microscopically the picture is that of actively blood-forming marrow. The fat in most instances is entirely replaced by marrow cells. Phagocytic endothelial cells are present in increased numbers and contain trypanosome remains, red blood corpuscles and myelocytes.

Trypanosomes are present in the blood sinuses and occasionally between the marrow cells. Trypanosome remains are usually abundant in endothelial cells. Round or oval deeply blue stained forms with nucleus and blepharoplast in endothelial cells are rather more common than in other tissues. In the case of a guinea-pig inoculated with T. brucei (November 1, and killed December 22, 1911), there are enormous numbers of trypanosome nuclei and blepharoplasts lying embedded in finely granular purplish staining material in masses among the marrow cells. Many of the nuclei are divided into two biscuit-shaped fragments (Fig. 8).

In contact film ("klatsch") preparations made and stained at the time of the autopsy large numbers of poorly stained flagella are found in the granular material containing the nuclei, so that it is almost certain that these nuclei are the remains of dead trypanosomes.

Skin: The gross changes in the six guinea-pigs infected with T. gambiense which showed lesions have been described above. The three studied microscopically showed identical processes and distribution of trypanosomes, and these were practically the same in skin taken from the region of the eyes, the perineum, back, and the ears. The skin of the monkey which showed external lesions is excluded from this description, because examination showed a phlegmonous

condition with great numbers of streptococci in the edematous areas and within polymorphonuclear leucocytes. Trypanosomes were, however, fairly numerous in the corium and subcutaneous tissue.

The sections of the skin and subcutaneous tissues show lesions involving the corium, subcutaneous fat and areolar tissue and muscle panniculus (Figs. 4, 5, and 6). Changes which are present in the epidermis are secondary in all probability to the lesions of the corium. The epidermis is thinned in places, and there is a general decrease in the thickness of the horny layer. The palisade layer is poorly defined as the cells have no regular arrangement, and mitotic epithelial cells are very numerous. There are occasional small down-growths into edematous and infiltrated foci of the corium.

The corium is infiltrated with large numbers of endothelial, lymphoid and plasma cells. In the earlier lesions there are evidences of edema in the separation of collagen fibrils and presence of a delicate fibrin reticulum. In older lesions, where there has been complete loss of hair, there are no evidences of edema; the connective tissue is compact and giant cells, singly and in miliary sized groups, are common (Fig. 4). These groups of giant cells and large endothelial cells have a close resemblance to the cutaneous lesions of kala-azar, a resemblance which is heightened by the leishmania-like appearance of the inclusions of trypanosome nuclei and blepharoplasts. Other stages in the process show large numbers of endothelial cells separating the bundles of collagenous fibers and scattered among them are occasional fibroblasts (Fig. 14). In the older lesions there is complete absence of the hair follicles and appendages. Hyaline remains of the arrector pili muscles are found in groups of endothelial cells.

The subcutaneous fat tissue is infiltrated with endothelial cells, lymphoid cells, and plasma cells. The fat cells are separated by edema in early lesions, and by fibroblasts and endothelial cells in lesions of long duration. There are many giant cells lining spaces once occupied by fat and often

completely filling the fat cell spaces. In the subcutaneous areolar tissue and fat tissue there are areas densely packed with endothelial cells which also contain many young fibroblasts (Fig. 5).

The fibers of the muscle panniculus and deeper muscles of the back are separated by edema or by infiltrative cells consisting of endothelial, lymphoid, and plasma cells (Fig. 6). Many of the muscle fibers have undergone hyaline change and are partially or completely destroyed by phagocytosis by endothelial cells and giant cells. Fibroblasts are common where the degeneration and phagocytosis of muscle fibers are most marked. The absence of polymorphonuclear leucocytes and granular leucocytes is a striking feature of the skin lesions except where there has been ulceration.

Trypanosomes are found in enormous numbers in the corium (Figs. 23 and 25), both in edematous areas and in areas tightly packed with endothelial cells and fibroblasts. They are abundant in the lesions of fat and muscle. They are often tightly packed in lymphatics, but most lie between the bundles of collagenous fibers or within the bundles amongst the fibrils. They cannot be found in the epithelium. Trypanosome nuclei and blepharoplasts are common in endothelial and giant cells, and rarely these cells contain a round or oval form with densely stained blue cytoplasm, nucleus, and blepharoplast.

Central nervous system: The brains of three monkeys and four guinea-pigs all infected with T. gambiense were examined. Gross lesions were seen in the case of a monkey inoculated with T. gambiense on December sixteenth, 1911, and killed on February ninth, 1912. There was slight flattening of the convolutions and opacity of the pia-arachnoid over the cisternæ at the base of the brain. This monkey during the last three days showed evidences of cerebral involvement, possibly due to increased intracranial pressure. There was tremor, photophobia, evidence of pain, and drowsiness. The monkey maintained the sleeping posture during this period; when roused it would eat, but would

promptly fall asleep again. Its behavior was markedly different from that of another T. gambiense monkey inoculated on the same date, but killed on January eleventh because of its moribund condition. The third T. gambiense monkey inoculated April first, and killed May fourth, 1912, developed external lesions, which have been described above. The animal gave evidences of pain and during the last two days remained in the sleeping posture. The clinical picture of this case was complicated by the phlegmon of the face.

The only cases that showed microscopic lesions were two monkeys inoculated on December sixteenth. The blood of these monkeys contained many trypanosomes when killed. Lesions in the brain of the monkey killed on January eleventh are very slight and consist of focal infiltrations of the meninges and about an occasional vessel in the brain with endothelial and lymphoid cells These lesions were found only in sections from the neighborhood of the sulcus cruciatus, though sections from both poles of the hemispheres, cerebellum, and cord were examined.

Trypanosomes in this case were rare but were found in the lesions of the pia-arachnoid, in the substance of the brain just beneath the pia, and in the perivascular spaces and in the adventitia of blood vessels of the brain.

The lesions in the brain of the monkey killed on February ninth were focal in character, but were more widely distributed over the brain than in the first monkey. These were found in sections from several different areas of the cerebrum and at the base of the brain in the pia covering the pons. The lesions consist of infiltrations of the pia-arachnoid with endothelial and lymphoid cells, an increase of the subpial neuroglia, and a few perivascular infiltrations of endothelial and lymphoid cells. A rare plasma cell also occurs in the infiltrations.

The infiltration of the meninges is most marked about blood vessels. The earliest lesions consist only of collections of endothelial cells. These cells are phagocytic and may

contain inclusions of trypanosome nuclei and, in older lesions, lymphoid cells. The endothelial cells are found in the substance of the brain for short distances beneath the pia and around blood vessels. The increase of neuroglia occurs in the form of clumps of neuroglia cells with abundant cytoplasm and many fibrils, grouped just beneath the pia, in regions where the infiltration is most marked and trypanosomes are most abundant. One mitotic neuroglia cell was found (Fig. 33).

The spinal cord was not sectioned in this case. Sections of the cerebellum show no lesions.

Trypanosomes were found in considerable numbers in infiltrated areas in the pia-arachnoid, in the subpial neuroglia tissue, in the perivascular infiltrations, in perivascular spaces not infiltrated, in the walls of small blood vessels and capillaries, and in foci scattered throughout the substance of the brain. In one instance a trypanosome was found in a ganglion (pyramidal) cell (Fig. 36). In the substance of the brain they were most numerous in the neighborhood of blood vessels. The most marked lesions and greatest number of trypanosomes occur in the region of the sulcus cruciatus (Figs. 31, 32, 33, 34, 35, 36, and 37).

Morphology of trypanosomes in tissues. — We have not attempted in the preparation of this paper to make an exhaustive study of the morphology of trypanosomes in tissues; and shall note briefly our observations made in the course of the examinations of the tissues for the presence of lesions and distribution of trypanosomes. With a view to a careful morphological study, contact (Klatsch) preparations stained with Giemsa's stain were made from all organs, including bone marrow of each animal, at the time of autopsy. This method gives better results than the ordinary one of making smears, as distortion of cells and trypanosomes is less liable. It necessitates using a large number of slides for each organ, as repeated contacts with a cut surface are necessary to obtain impressions sufficiently free from blood. In the case of bone marrow, light contact will generally

secure a good spread of cells at the periphery of the impression. These preparations have been studied so far only with the view of controlling the finding in sections.

By far the greatest number of trypanosomes seen in sections and in the film (contact) preparations are normal and dividing flagellate forms. These were found lying in tissue spaces, between cells and between collagen fibrils of connective tissue. Occasional flagellate forms in phagocytes were seen.

The interpretation of the abundant leishmania-like forms in phagocytic cells and in the spleen and bone marrow in masses (Figs. 7 and 8) presents difficulties in sections. The control film preparations have always confirmed the absence of the cytoplasm observed in sections. This fact, together with the finding of a series of forms intermediate between the well-preserved nucleus and blepharoplast in a small vacuole and the irregularly-shaped inclusions with chromatin staining reaction, have led us to conclude that most of these forms are stages in the destruction of trypanosomes.

It is probable that other forms, which are round or oval in shape, and which consist of a deeply stained blue cytoplasm, nucleus, and blepharoplast, are the resistant forms described first by Moore and Breinl. Fantham [13] has recently contributed important evidence to the effect that these forms are viable and may develop into the flagellate forms.

We have found them within phagocytic endothelial cells (Figs. 15 and 19). They are most abundant in lymph nodes, spleen, bone marrow, and lung. A few were found in lesions in all organs, including the skin. The ability to distinguish, in sections, *a*, intracellular forms which, though resembling Leishman's bodies, are plainly the remains of trypanosomes undergoing destruction, and *b*, forms which have all the structural features essential to viability, is additional evidence for the existence of latent or resistant forms of T. brucei and T. gambiense.

The flagellate forms show clearly the various stages of division that are shown in film preparations. In lesions, T. gambiense often shows a vacuole posterior to the nucleus

(Fig. 30) and, rarely, one or two deeply stained red granules are present in this vacuole. Multiple division forms were not seen.

DISCUSSION AND SUMMARY. — We have not been able to make any substantial contribution to the pathology of trypanosomiasis. The character of the cell reactions has been described by the authors quoted in the introduction and by others.

The production of extensive skin lesions with giant cell formation in guinea-pigs infected with T. gambiense is of interest. The abundance of trypanosomes in the lesions of these guinea-pigs and scarcity in the blood is similar to Yorke's[6] finding in the case of T. rhodesiense infected goats.

The initial cell reaction is the proliferation of endothelial cells. The finding of numerous intravascular mitoses of endothelial cells in lung, liver, spleen, and kidney indicates the source of the increase of large mononuclear leucocytes of the blood in human trypanosomiasis.[14]

The sequence of pathological change is invasion of tissues by trypanosomes, edema (not constant), endothelial cell infiltration, lymphoid and plasma cell infiltration, and fibrosis. Fibrosis occurs in the walls of the alveoli of the lungs and in glomeruli of the kidneys, after hyaline degeneration of the endothelial cells, which have blocked the vessels, has taken place. Giant cell formation and fibrosis occurs in the skin and subcutaneous fat.

In the brain subpial gliosis follows infiltration of the meninges.

The absence of granular leucocytes in all lesions is worthy of note.

Phagocytosis of trypanosomes takes place almost exclusively by endothelial cells. In two instances trypanosome nuclei were found in granular leucocytes (eosinophilic) of the myeloid type — in the bone marrow of a guinea-pig and in a lymph node of a white rat. Both animals were infected with T. brucei. In the peripheral sinus of the lymph node

of the rat the form within the granular leucocyte was of the latent or resistant type. These findings are consistent with those of v. Prowazek [15] and Sauerbeck.[4]

In all animals infected with T. brucei and T. gambiense, lesions have been found in practically every organ, and trypanosomes have been found in these lesions. The most common seats of lesions are the connective tissue structures, and trypanosomes are found more abundantly in fibrous connective tissue than in any other place before the occurrence of the lesions. This preference for connective tissue heightens the numerous analogies between syphilis and trypanosomiasis, as in the former the spirochetes, other than in congenital syphilis, are found most abundantly in connective tissue. The failure of trypanosomes to invade the epidermis in the T. gambiense guinea-pigs is in marked contrast to the behavior of the spirochetes of syphilis, yaws, and tropical ulcer.

The demonstration in two monkeys of trypanosomes in the perivascular lesions, in the lesions of the meninges, and migrating in the substance of the brain, is positive proof that in the central nervous system the lesions, as elsewhere, are due to the presence of trypanosomes.

We are well aware that a more careful daily examination of the number and morphology of trypanosomes in the blood of the animals used would have increased the value and scope of our observations. A larger series of animals would probably have thrown some light on certain vagaries in distribution of the lesions and the virulence of the strains observed. A more extensive study made on lines similar to the present would definitely settle the question of the fate of trypanosomes during the stage of decrease in the blood stream. While we have demonstrated the stages in the destruction of trypanosomes in the tissues within cells and en masse in the spleen and bone marrow, we have not correlated these findings with the variation in numbers of trypanosomes in the blood stream. It is not, however, necessary to assume, as Fantham's [13] conclusions indicate, that a change of form accompanies the decrease of trypanosomes

in the blood, as we have shown that the vast majority of trypanosomes in the tissues are flagellate forms.

The character of the initial cell reactions, endothelial and lymphocyte, and the absence of polymorphonuclear and granular leucocytes — if comparisons with reactions to bacteria are valid — indicates a very low degree of toxicity of trypanosomes.

CONCLUSIONS.

In trypanosomiasis the trypanosomes do not remain confined to the blood vessels and lymphatics. They invade the connective tissue structures of all organs, the reticular tissue of lymph nodes and spleen, and the substance of the brain.

The lesions of trypanosomiasis are due to the presence of the trypanosomes in the tissues.

The most common form of trypanosome in tissue, and we believe the one most active in the production of lesions, is the flagellate form.

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DESCRIPTION OF PLATES.

PLATE V., FIG. 1. — Guinea-pig infected with Trypanosoma gambiense; duration, at time of photograph, November 1, 1911, to April 13, 1912. Shows lesions about the eyes, ears, and nose.

FIGS 2 and 3. — Guinea-pig infected with Trypanosoma gambiense; duration, at time of photograph, December 16, 1911, to April 18, 1912. Show lesions of back and genitalia respectively.

FIG. 4. — Photomicrograph at about 150 diameters of a section of the skin of back of a Trypanosoma gambiense infected guinea-pig; duration November 1, 1911, to March 29, 1912. Eosin-methylene-blue stain. Shows giant cells and infiltration in corium. See Figure 13, Plate VII., for higher power.

FIG. 5. — Photomicrograph at about 150 diameters, of a section of the subcutaneous fat from same case as Figure 4. Eosin-methylene-blue stain. Shows disappearance of fat, infiltration, fibrosis, and giant cells.

FIG. 6. — Photomicrograph at about 150 diameters, of a section of the muscle panniculus; from same case as Figures 4 and 5. Eosin-methylene-blue stain. Shows infiltration and fibrosis.

PLATE VI., FIG. 7. — Photomicrograph ot a section of the spleen oi a Trypanosoma brucei infected guinea-pig; duration December 16, 1911, to

January 13, 1912. Giemsa stain; 1,000 diameters. Shows a mass of trypanosome nuclei (agglomeration?), some of which are in endothelial cells, in the reticular tissue.

FIG. 8. — Photomicrograph of a section of the bone marrow of a Trypanosoma brucei infected guinea-pig; duration November 1 to December 22, 1911. Giemsa stain; 1,000 diameters. Shows masses of trypanosome nuclei (agglomeration?) embedded in granular material, probably derived from the cytoplasm of the trypanosomes. A few nuclei are in endothelial cells; a few are surrounded by deeply stained cytoplasm. Note resemblance to leishmania.

FIG. 9. — Photomicrograph of the lung of a Trypanosoma gambiense infected guinea-pig; duration October 6 to November 29, 1911. Magnification about 150 diameters; eosin-methylene-blue stain. Shows thickening of alveolar walls and filling of alveoli with endothelial and giant cells.

FIG. 10. — Same as Figure 9, from a different part of the lung. Shows thickening of alveolar walls due to endothelial cell proliferation. See Figure 22, Plate VIII.

PLATE VII., FIG. 11. — Photomicrograph of a section of the liver of a Trypanosoma brucei infected guinea-pig; duration November 1 to December 22, 1911. Giemsa stain; 1,000 diameters. Shows trypanosomes in infiltration about bile duct. In the lower left hand corner, it shows a lymphatic containing numerous trypanosomes.

FIG. 12. — Photomicrograph of a section of a lymph node of a Trypanosoma gambiense infected rat; duration December 28, 1911, to January 17, 1912. Giemsa stain; 1,000 diameters. Shows vacuolated endothelial cells which contain trypanosomes and trypanosome remains. There are also some free trypanosomes. The field is taken from a sinus packed with endothelial cells.

FIG. 13. — Photomicrograph of giant cells in a section of the skin from a Trypanosoma gambiense infected guinea-pig; duration November 1, 1911, to March 29, 1912. A high power of Figure 4. Eosin-methylene-blue stain; 1,000 diameters. Two of the giant cells contain inclusions, which are remnants of trypanosomes.

FIG. 14. — Skin; same case as Figures 12 and 4. From deep layer of corium. Phosphotungstic acid hematein stain; 1,000 diameters. Shows fibroblasts and endothelial cells; the latter are round or oval. There are also many nuclei of trypanosomes which are the small dark bodies scattered throughout the tissue, between cells and in collagen fibers. Near the top is a mitotic endothelial cell.

PLATE VIII., FIG. 15. — Photomicrograph of a section of the liver of a Trypanosoma brucei infected guinea-pig; duration December 16, 1911, to January 13, 1912. Giemsa stain; 1,000 diameters. Shows a giant cell, in a lymphatic of a portal space, filled with trypanosome remains. The larger sized oval body in the center is probably a "resistant" form.

FIG. 16. — Photomicrograph of a section of the lung of a Trypanosoma gambiense infected rat; duration December 30, 1911, to January 17, 1912. Giemsa stain; 1,000 diameters. Shows a trypanosome in the lesion of the

fat tissue about the great vessels and bronchus. The invading cells are endothelial cells.

FIG. 17. — Photomicrograph of a section of the kidney of a Trypanosoma gambiense infected rat; duration December 30, 1911, to January 17, 1912. Giemsa stain; 1,000 diameters. Shows a lesion, containing trypanosomes, at the edge of a glomerulus.

FIG. 18. — Photomicrograph of a section of the lung of a Trypanosoma gambiense infected monkey; duration December 16, 1911, to January 11, 1912. Giemsa stain; 1,000 diameters. Shows early infiltration and trypanosomes in the connective tissue surrounding a pulmonary artery.

FIG. 19. — Same as Figure 18. Shows an oval, "resistant" form in an endothelial cell in a capillary of an alveolar wall.

FIG. 20. — Photomicrograph of a section of the liver of a Trypanosoma lewisi infected white rat; duration April 13 to 20, 1912. Eosin-methylene-blue stain; 1,000 diameters. Shows a mitotic endothelial cell in a sinusoid.

FIG. 21. — Photomicrograph of a section of the kidney of a Trypanosoma gambiense infected monkey; duration December 16, 1911, to February 9, 1912. Eosin-methylene-blue stain; 1,000 diameters. Shows a mitotic endothelial cell in a capillary of a glomerular tuft; the capillaries of this tuft are nearly wholly occluded by endothelial cells.

FIG. 22. — Photomicrograph of a section of the lung of a Trypanosoma gambiense infected monkey; duration December 16, 1911, to February 9, 1912. Eosin-methylene-blue stain; 1,000 diameters. Shows two mitotic endothelial cells in a capillary of an alveolar wall.

PLATE IX., FIG. 23. — Photomicrograph of a section of the skin of the back of a Trypanosoma gambiense infected guinea-pig; duration November 1, 1911, to March 29, 1912. Giemsa stain; 500 diameters. Shows distribution of trypanosomes in corium, endothelial cell infiltration, and loss of appendages of the epidermis. See Figure 25 for higher power.

FIG 24. — Photomicrograph of a section of the heart of the naturally infected (Trypanosoma dimorphon) Ayrshire bull Giemsa stain; 1,000 diameters. Shows infiltration of an intramuscular septum, trypanosomes, and trypanosome nuclei. A single trypanosome is well shown between the muscle fibers on the left. See Figure 28.

PLATE X., FIG. 25. — Same as Figure 23, Plate IX. One thousand diameters.

FIG. 26. — Photomicrograph of a section of the splenic attachment of a Trypanosoma gambiense infected monkey; duration December 16, 1911, to February 9, 1912. Shows trypanosomes in edematous infiltrated connective tissue at the hilum of the spleen.

FIG. 27. — Photomicrograph of a section of the colon of a Trypanosoma gambiense infected white rat; duration December 30 1911, to January 17, 1912. Shows the lesion and presence of trypanosomes in the submucosa of the colon. The invading cells are mostly endothelial cells.

FIG. 28. — Same as Figure 24. Shows the lesion and presence of trypanosomes in the endocardium. The cells are mostly endothelial cells.

FIG. 29. — Photomicrograph of a section of the liver of a Trypanosoma gambiense infected white rat; duration December 30, 1911, to January 13, 1912. Giemsa stain; 1,000 diameters. Shows endothelial cells in sinusoids. The upper cell contains a trypanosome nucleus with a trace of cytoplasm attached. The lower cell has several trypanosomes attached.

FIG. 30. — Photomicrograph of a section of the lung of a Trypanosoma gambiense infected white rat; duration November 29 to December 8, 1911. Giemsa stain; 1,000 diameters. Shows the lesion and presence of trypanosomes in the connective tissue around a large pulmonary vein.

PLATE XI. — These figures are all from the cerebrum of a monkey infected with Trypanosoma gambiense; duration December 16, 1911, to February 9, 1912

FIG. 31. — Drawing. Zeiss apo. obj. 1.5 millimeters, comp. ocular six, with camera lucida tracing upon the desk top. Shows a trypanosome near a small vein which enters the pia mater. The closely packed dark nuclei are endothelial cell nuclei. From a Giemsa stained section.

FIG. 32. — Drawing. Same as Figure 31. A field of the cortex about five millimeters below the pia. Shows the number of trypanosomes that may be found in a single oil immersion field. In the upper right hand corner a trypanosome lies partly in the space about a capillary and partly in the brain tissue. From a Giemsa stained section.

FIG. 33. — Photomicrograph of a Giemsa stained section; 1,000 diameters. Shows a trypanosome in the infiltrated pia, one in the subpial neuroglia, and a third deeper in the brain tissue. The cell above and to the right of the trypanosome beneath the pia is a mitotic neuroglia cell, only partly in focus.

FIG. 34. — Photomicrograph of an eosin-methylene-blue stained section; 1,000 diameters. Shows trypanosomes about a small vessel of the cortex.

FIGS. 35 and 37. — Photomicrographs of a Giemsa stained section; 1,000 diameters. Each shows a trypanosome deep in the cortex.

FIG. 36. — Photomicrograph of a Giemsa-stained section; 1,000 diameters. Shows a trypanosome in a ganglion cell of the cortex.

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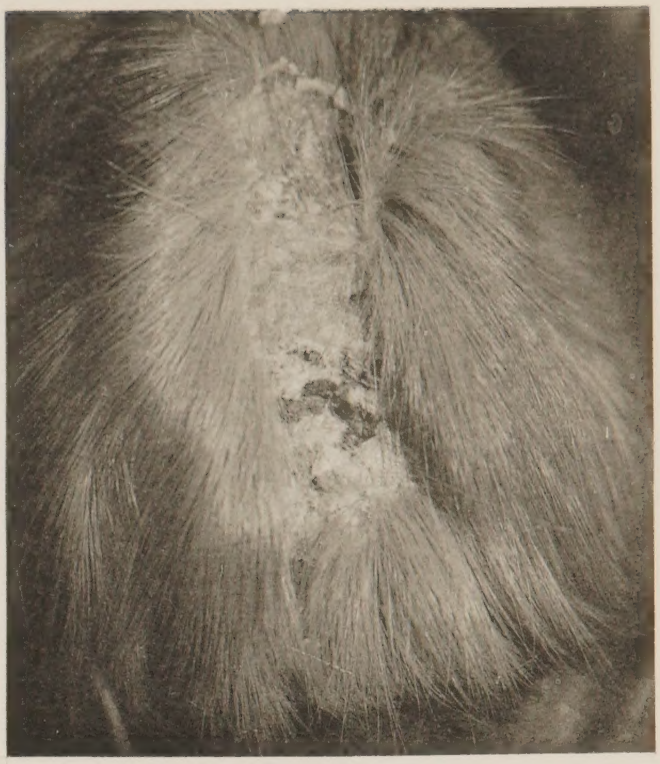

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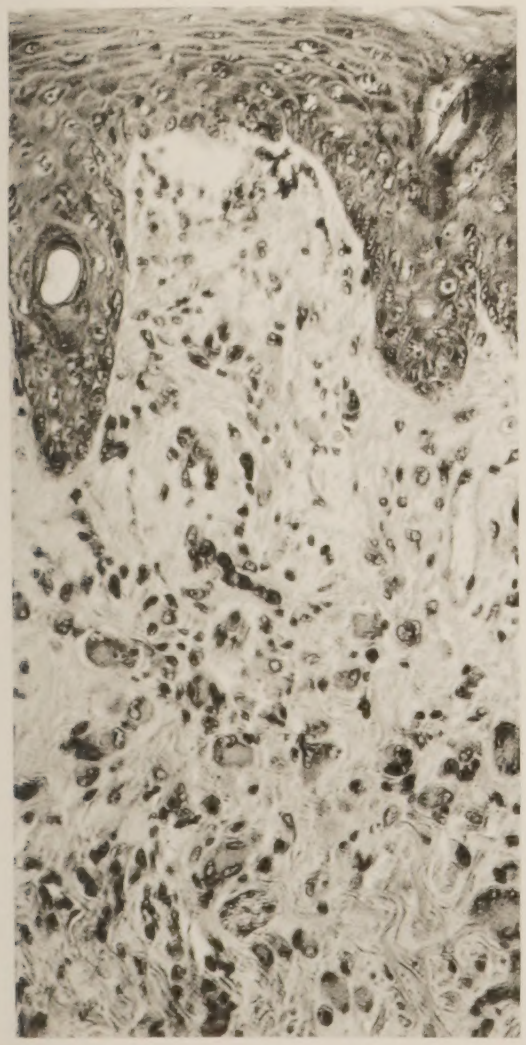

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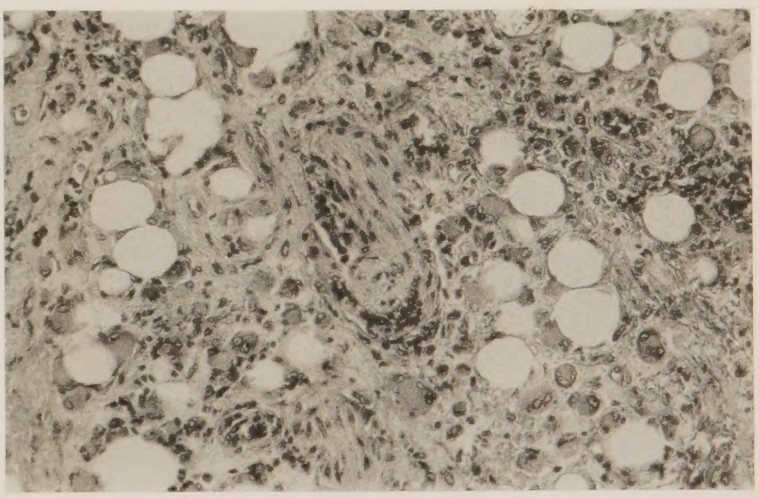

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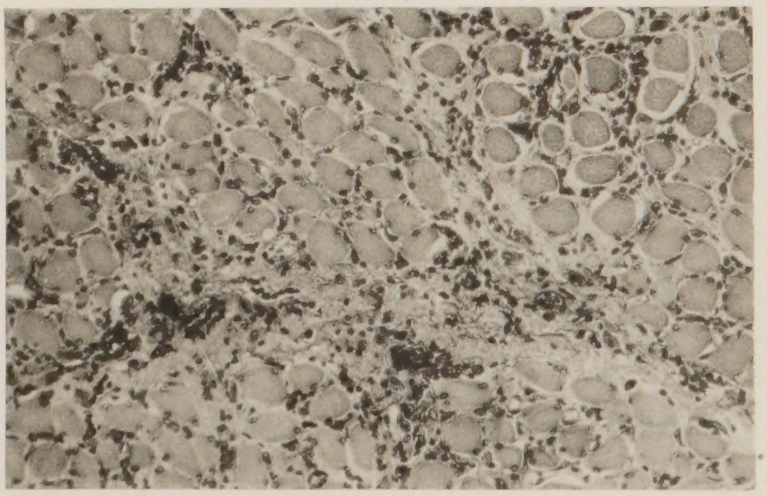

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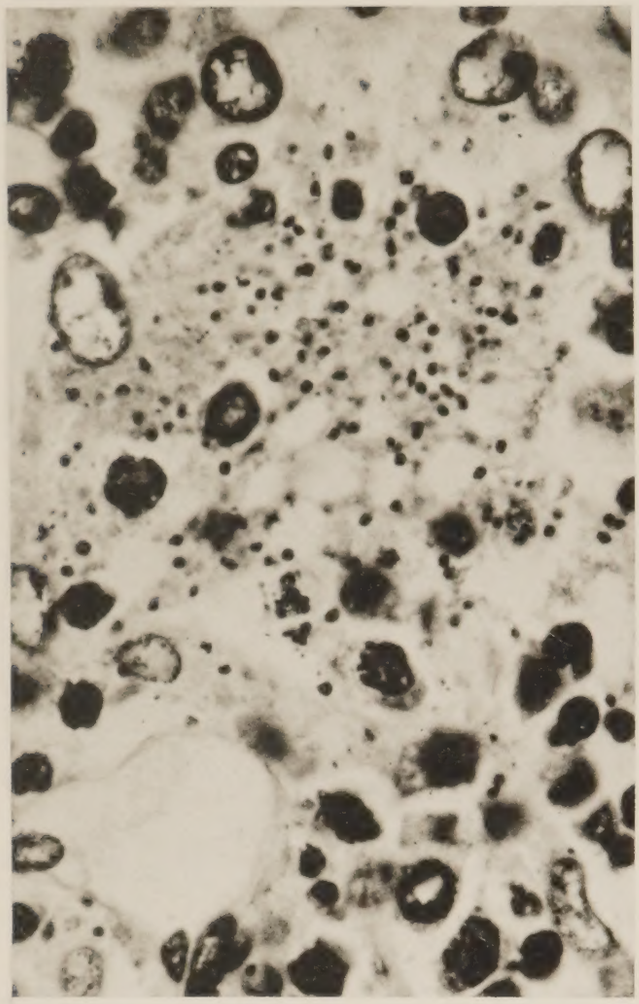

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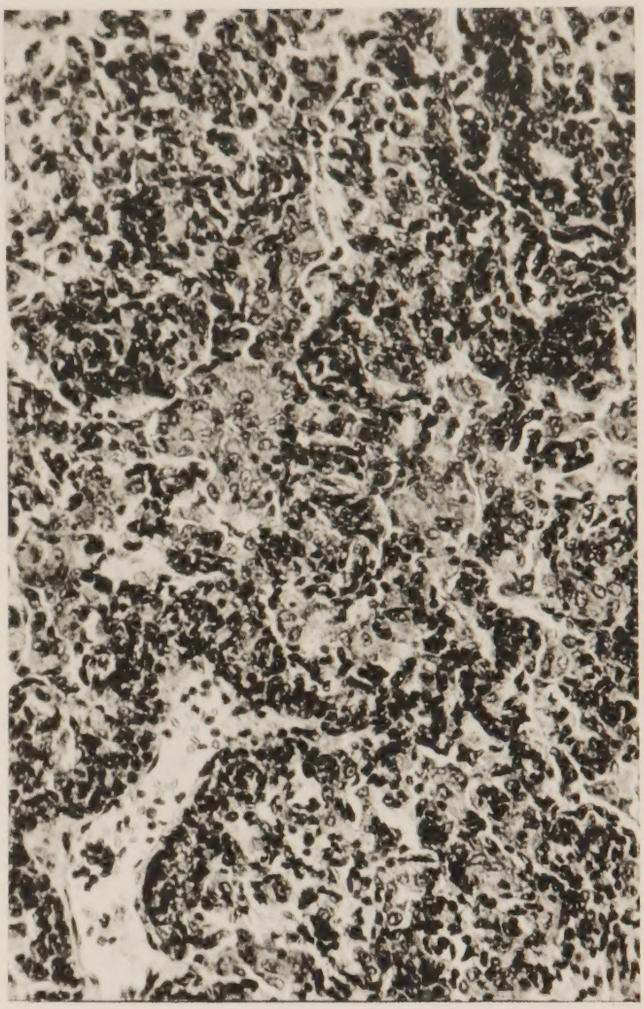

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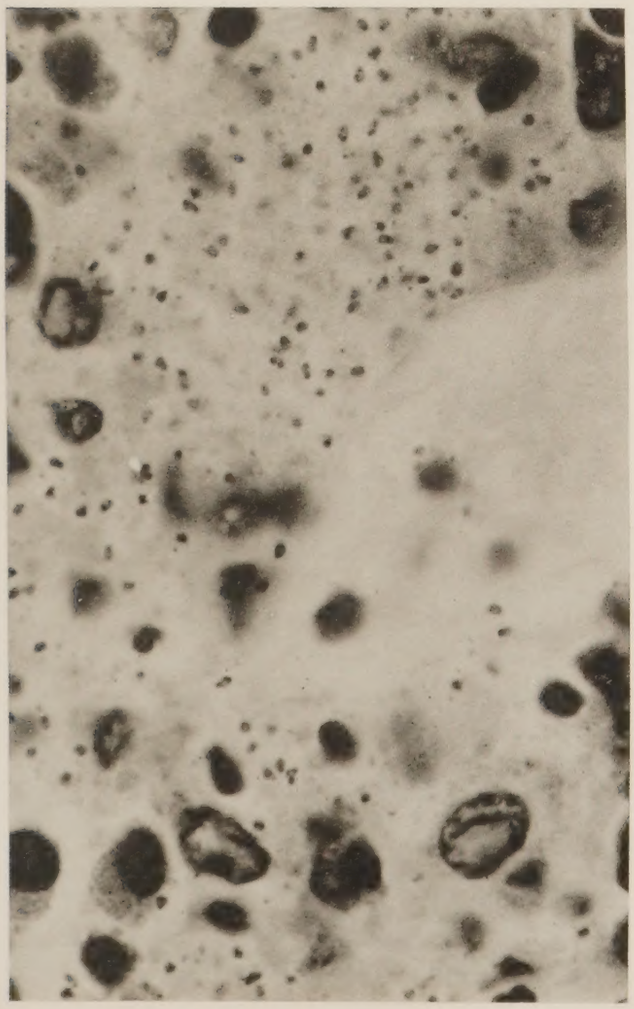

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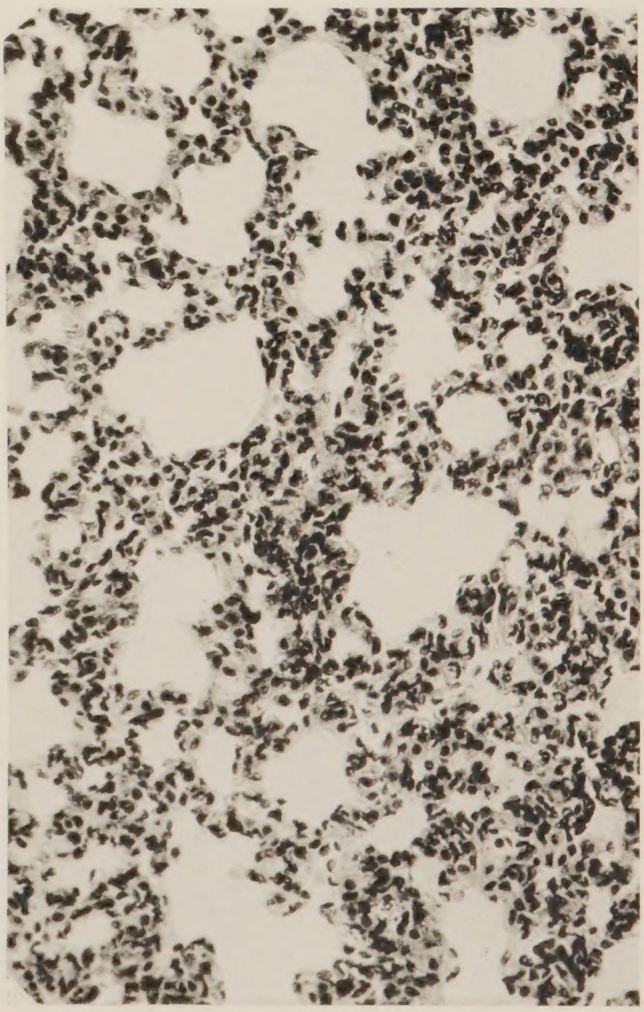

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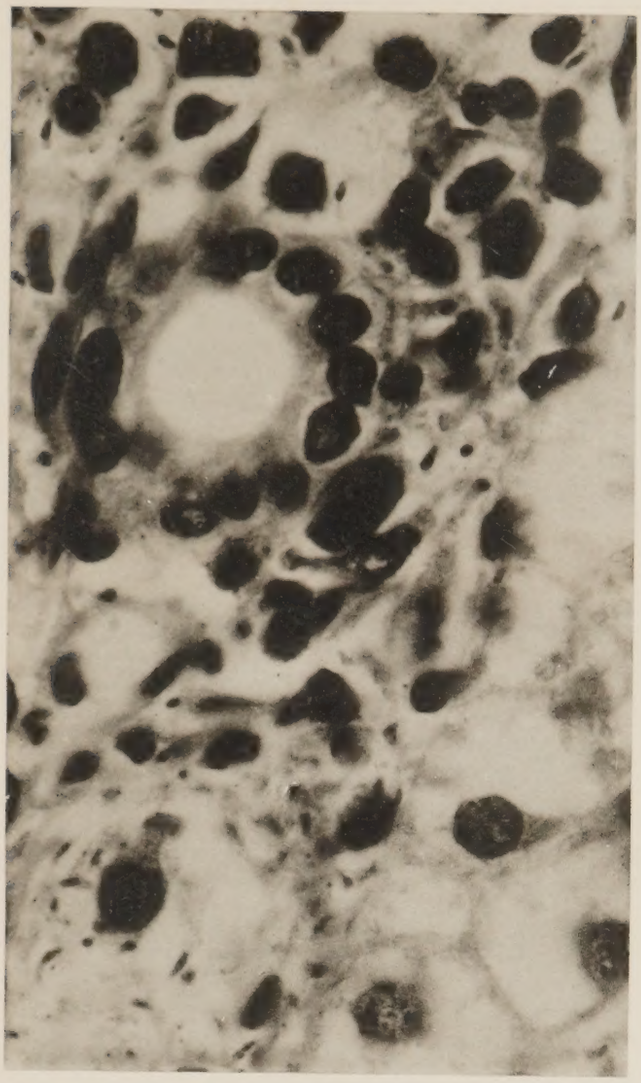

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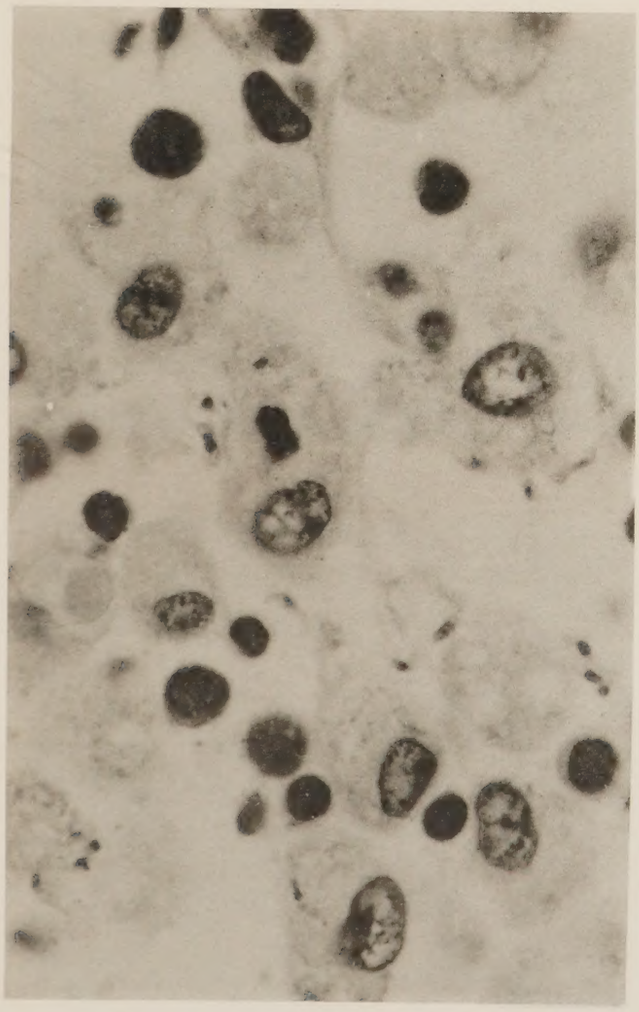

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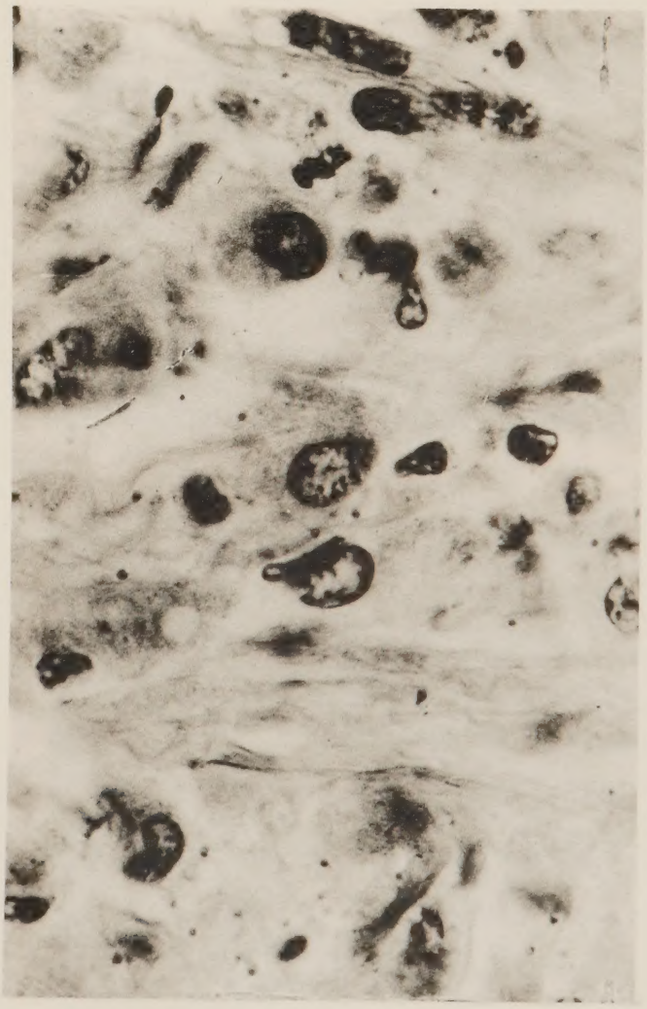

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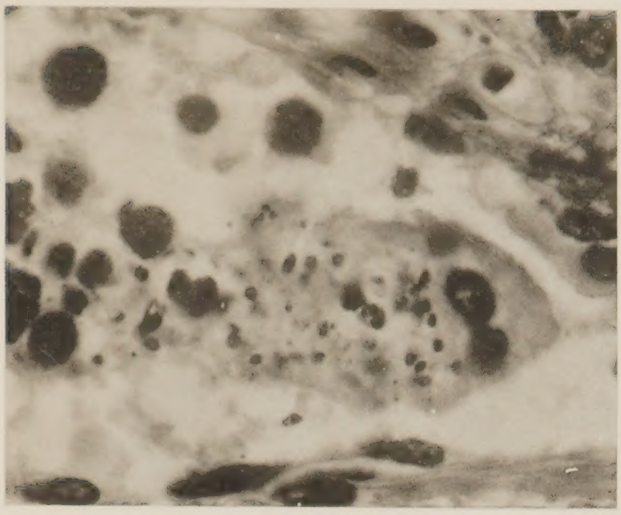

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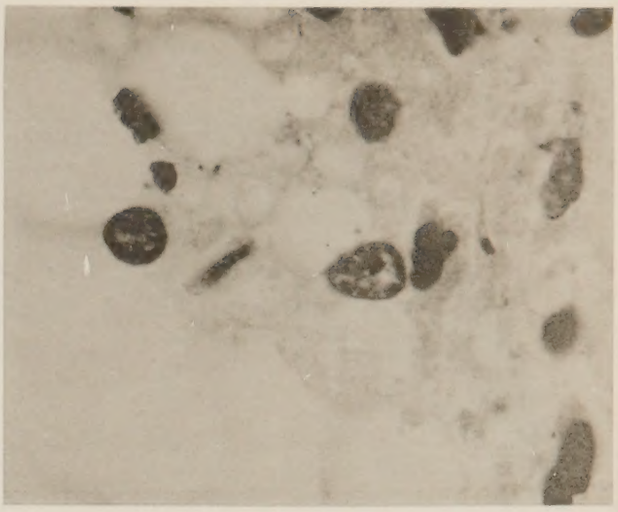

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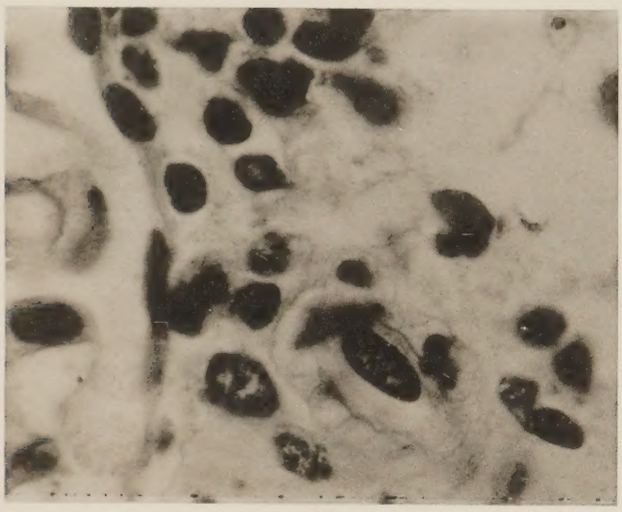

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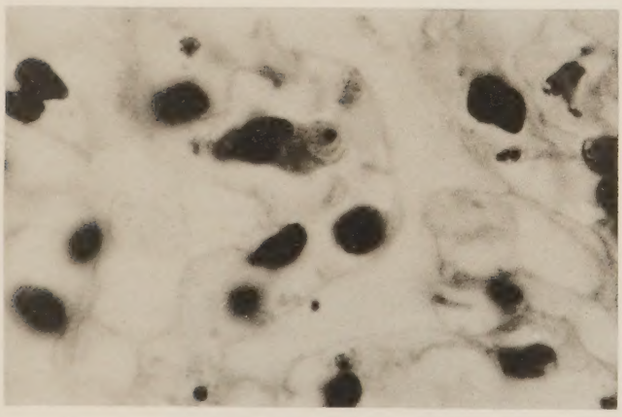

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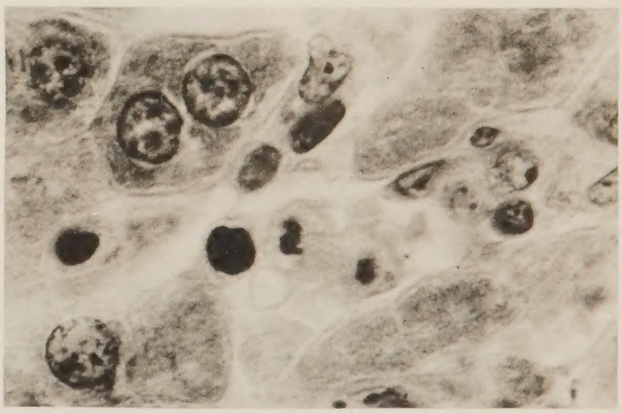

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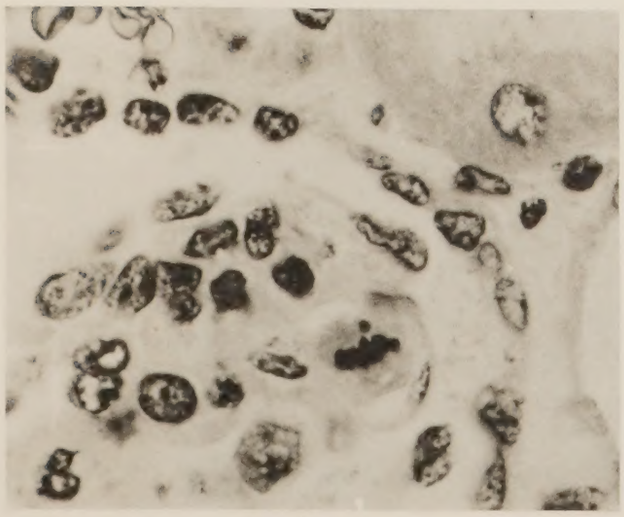

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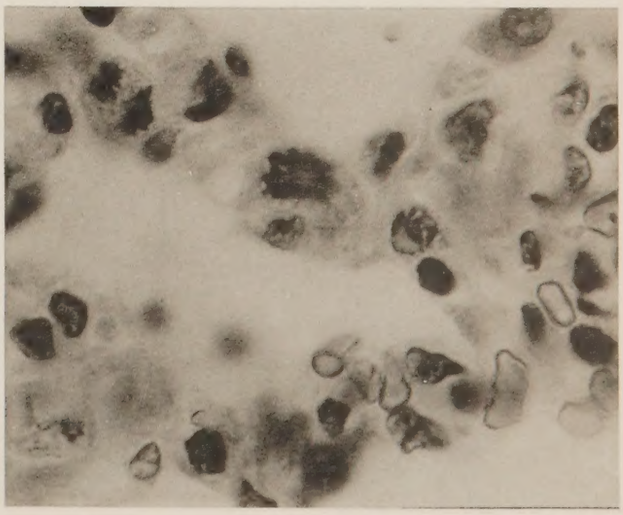

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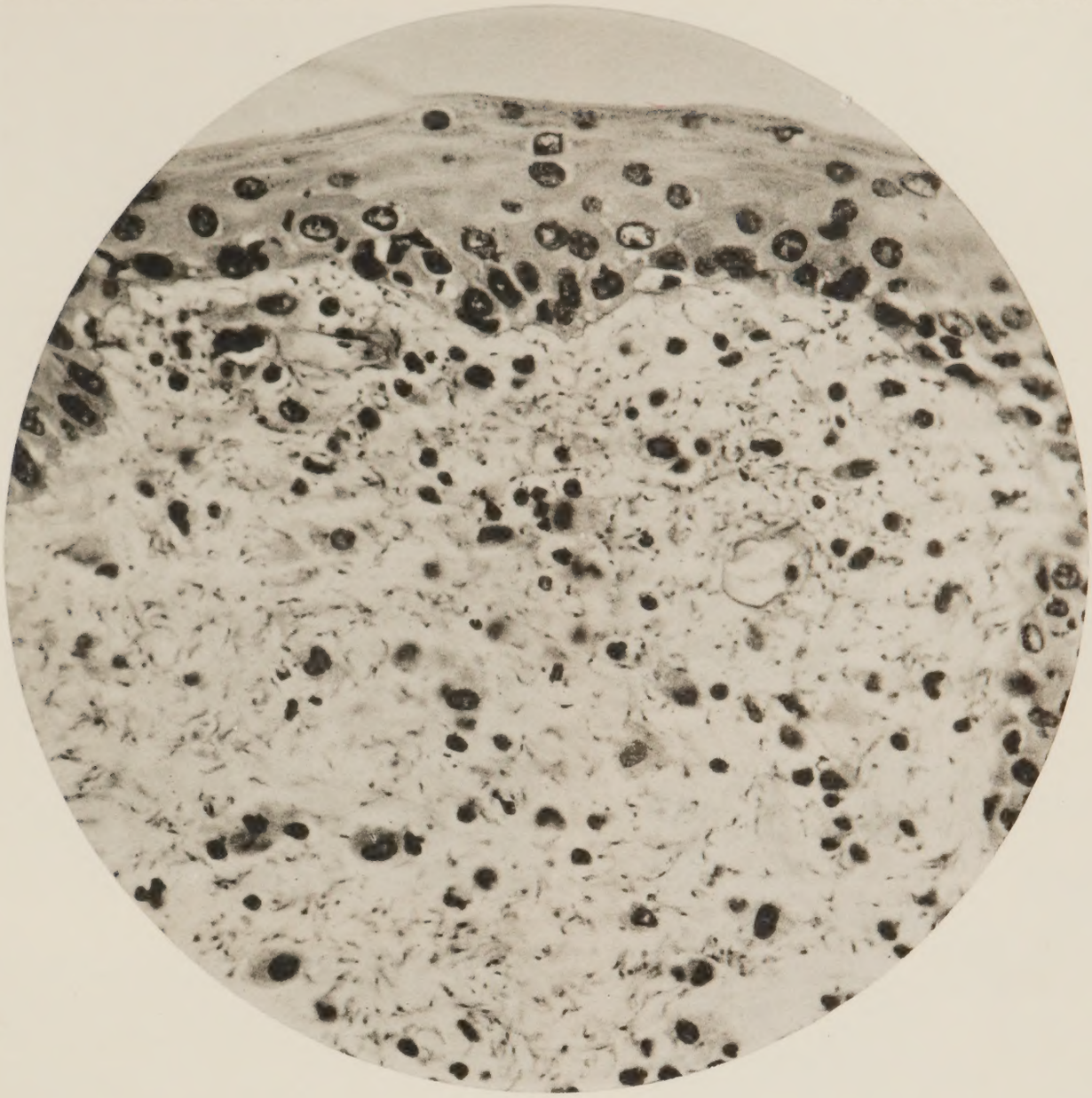

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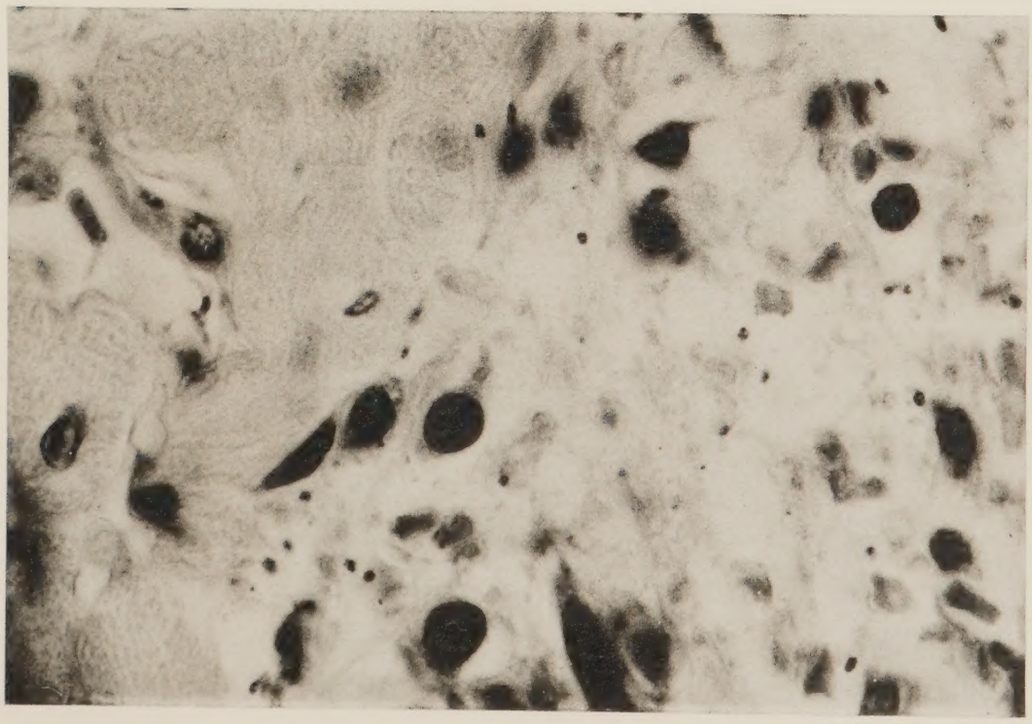

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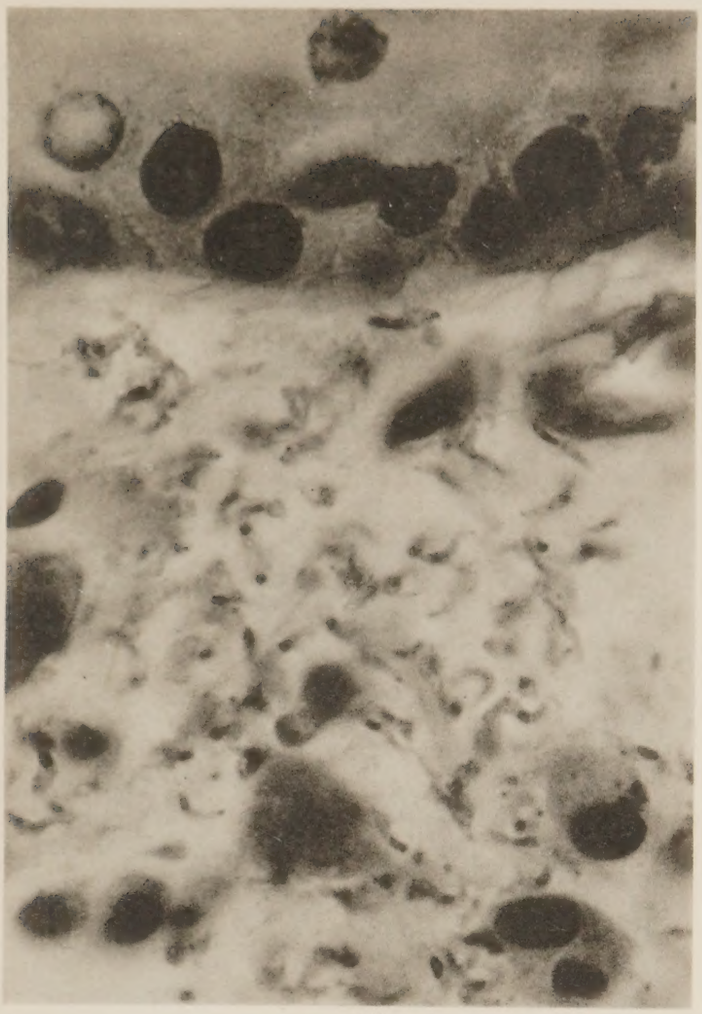

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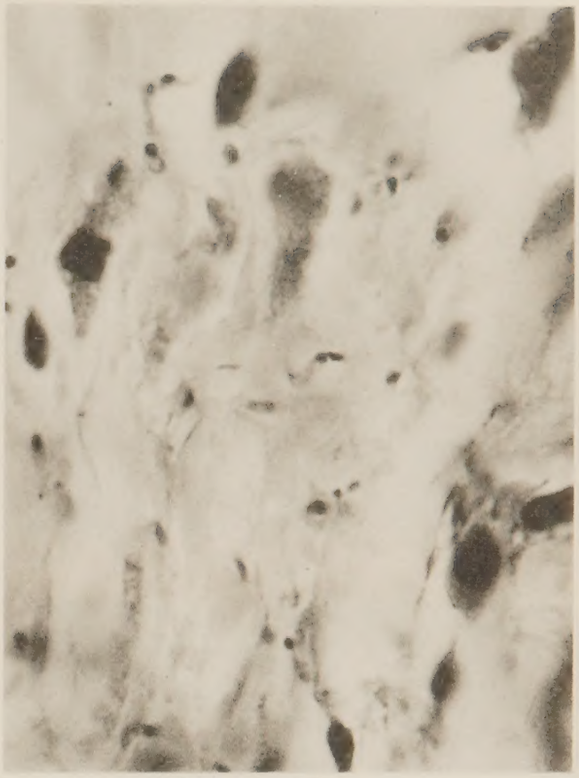

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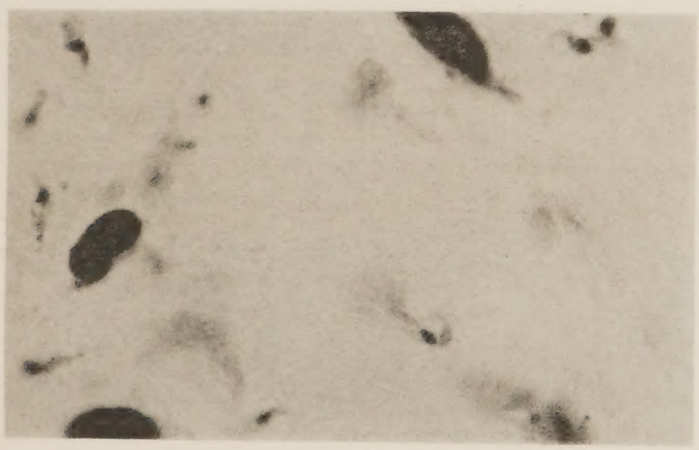

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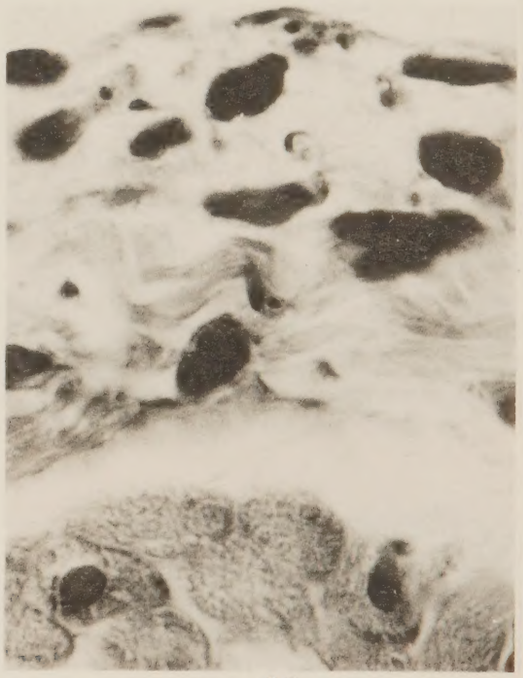

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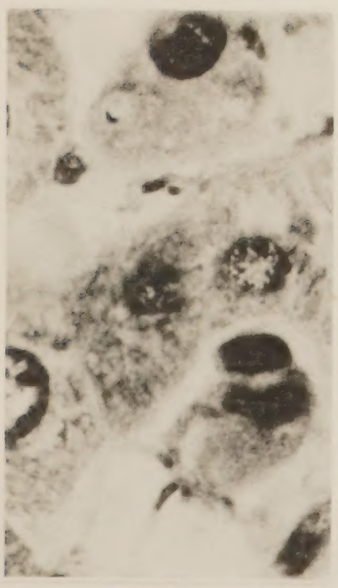

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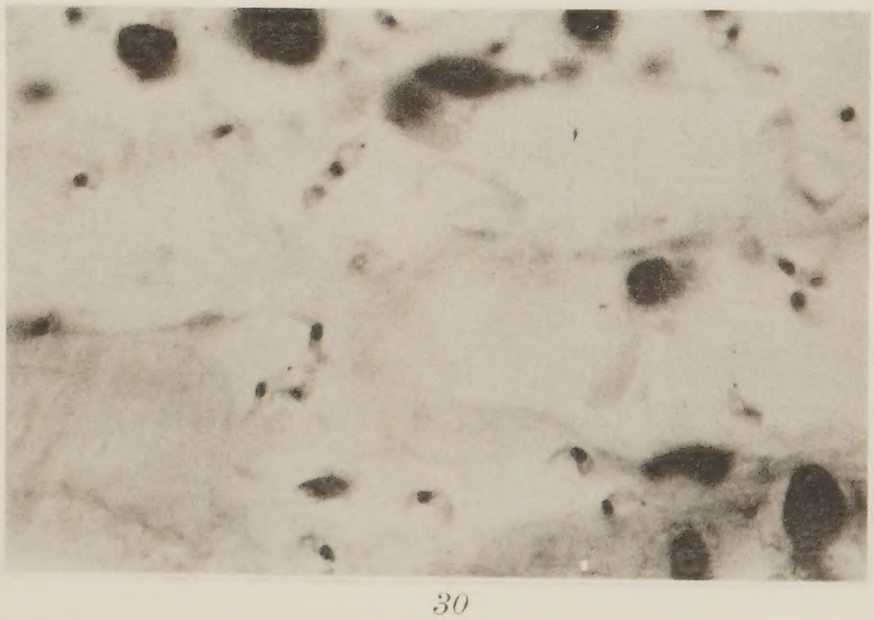

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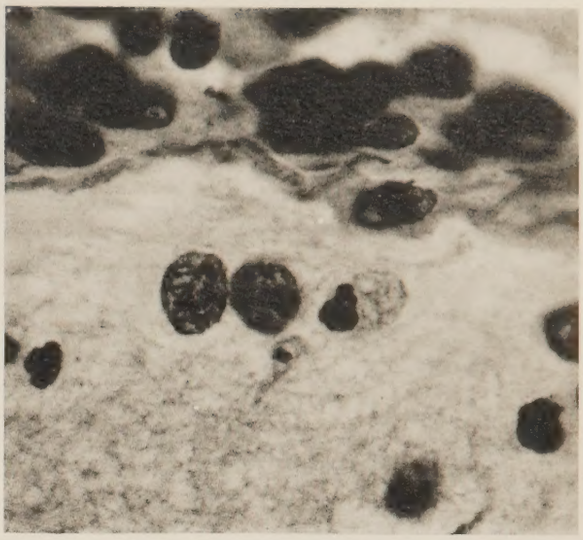

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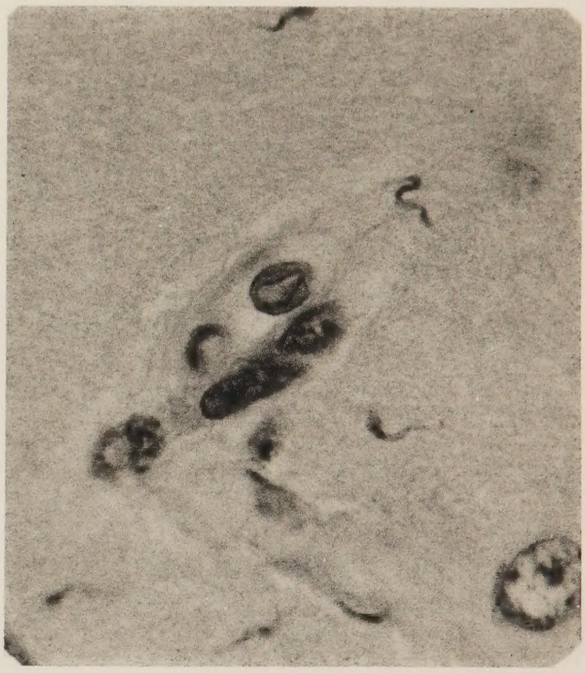

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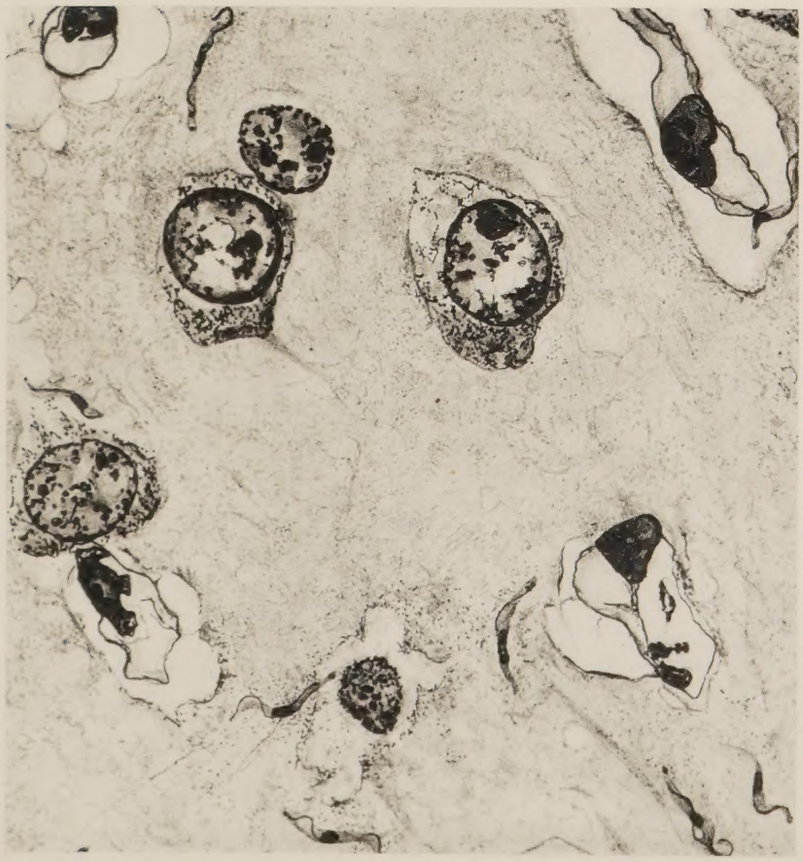

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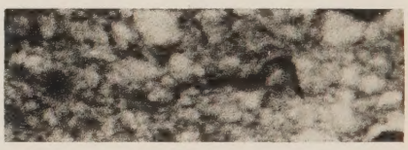

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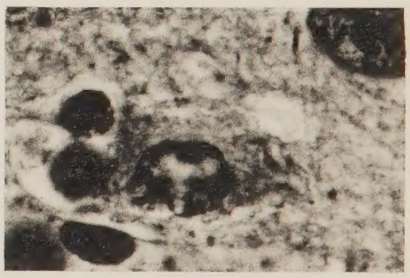

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